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THE FATE OF CAFFEINE IN MAN AND A METHOD FOR ITS ESTIMATION IN BIOLOGICAL MATERIAL

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Caffeine is extensively used as a therapeutic agent and is widely ingested in the form of caffeine containing beverages, yet little is known concerning its fate in the body. The development of a simple and sensitive method for the estimation of caffeine in biological materials has permitted studies of its absorption, excretion, distribution and rate of biotransformation in man.

CHEMICAL METHOD. *The estimation of caffeine.* Older methods for the determination of caffeine in biological material involve extraction into chloroform, followed by assay of the drug by its nitrogen content (Kunz, 1935) or by a color-producing murexide reaction (Tanaka and Ohkubo, 1937). These methods are insensitive and inaccurate. Recently, Ishler *et al.* (1948) described a sensitive method, using ultraviolet spectrophotometry, for the estimation of caffeine in coffee preparations. This method was applied by Fisher *et al.* (1949) to the estimation of caffeine in urine. In this method caffeine is extracted from the biological material with chloroform, which also extracts other methyl xanthines and interfering normal constituents of biological materials.

In the following procedure, caffeine is isolated from biological material at pH 7-8 by extraction into benzene. The compound is then returned to an aqueous phase by shaking with 5 *N* HCl and its absorption in the ultraviolet is measured at 273 mμ. The relatively nonpolar solvent benzene was chosen as the solvent least likely to extract metabolic products of caffeine and normal constituents of urine according to principles previous described (Brodie *et al.*, 1947). The extraction is augmented by saturating the aqueous phase with sodium chloride. A small amount of interfering blank material in urine and tissues which is also extracted into benzene is almost entirely removed by shaking the solvent extract with dilute NaOH. Since caffeine is basic it can be extracted from the benzene phase into 5 *N* HCl. This effectively separates it from methylated xanthines such as theobromine and theophylline.

Procedure for plasma. Pipet 1 to 5 ml. of plasma into a 60 ml. glass-stoppered bottle containing 40 ml. of benzene[2] and about 3 gm. of sodium chloride. Shake for 20 minutes. Centrifuge the bottle and pipet 30 ml. of the benzene phase into a 60 ml. glass-stoppered bottle[3] containing 4 ml. of 5 *N* HCl. Shake for 5 minutes, transfer 3 ml. of the acid phase to a quartz cuvet. Determine the optical density at 273 mμ in a Beckman DU spectrophotometer.[4]

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[2] Benzene, reagent grade, is purified by successive washings with ⅕ volume of 1 *N* NaOH, 1 *N* HCl, and twice with water.

[3] Glassware used in this and subsequent steps in the chemical procedure is immersed in 5 *N* HCl for 2 hours and thoroughly rinsed with distilled water. The quartz cuvets are similarly treated.

[4] The optical density reading of 5 *N* HCl in a quartz cuvet slowly increases with time. To minimize this potential error, the optical density readings should be measured within a few minutes after the extraction of the caffeine into the acid phase.

The instrument is set at 100 per cent transmission with distilled water. An optical density of about 0.200 is obtained when 25 microgm. of caffeine are assayed by this procedure. Standards are prepared by dilution of a stock caffeine solution with 5 *N* HCl.

Urine and tissues. Pipet 3 ml. of urine or tissue homogenate,[5] adjusted to pH 7–8, into a 60 ml. glass-stoppered bottle containing 40 ml. of benzene and about 3 gm. of sodium chloride. Shake for 20 minutes. Centrifuge the bottle and transfer as much of the benzene phase as possible to a 60 ml. glass-stoppered bottle containing 3 ml. of 0.1 *N* NaOH saturated with sodium chloride. Shake for 5 minutes and centrifuge. Pipet 30 ml. of the benzene phase into a 60 ml. glass-stoppered bottle containing 4 ml. of 5 *N* HCl and continue as described for the estimation of caffeine in plasma.

Caffeine added to biological material in amounts from 5 to 50 microgm. were recovered with adequate precision (98 ± 3 per cent).

Appraisal of specificity. There is a small blank equivalent to 0.1–0.5 microgm. of caffeine per ml. of normal plasma and 0.5 to 2.0 microgm. per ml. of urine or per gram of organ tissue. The possible interference of metabolic products of the drug in the plasma procedure was examined by the technique of comparative distribution ratios (Brodie *et al.*, 1947). Plasma was obtained two hours after 7 mgm. per kgm. of caffeine was administered intravenously to two human subjects. The apparent caffeine was extracted from the pooled plasma in the same manner as described in the procedure. The distribution ratios of the apparent caffeine were compared with those of authentic caffeine in a series of two phase systems consisting of water at pH 7 and benzene and petroleum ether in various proportions. The results indicate that the two substances possess the same solubility characteristics and were presumably the same compound (table 1).

TABLE 1

Distribution of caffeine and apparent caffeine between water and various benzene-petroleum ether mixtures

The apparent caffeine from the pooled plasma of two subjects receiving caffeine was extracted into benzene as described under the chemical method. The drug was returned to 5 *N* HCl. The acid extract was adjusted with NaOH to pH 7. Aliquots of this solution and of an authentic solution of caffeine similarly adjusted to pH 7 were shaken with 4 volumes of the various benzene petroleum ether mixtures. The distribution is expressed as the ratio of the amount of compound in the organic phase to total compound.

PER CENT PETROLEUM ETHER IN BENZENE	APPARENT CAFFEINE FROM PLASMA	AUTHENTIC CAFFEINE
0	0.89	0.92
25	0.81	0.84
50	0.60	0.62
63	0.45	0.46
75	0.27	0.27

EXPERIMENTAL. *Urinary excretion of caffeine.* Three male subjects each received 500 mgm. of caffeine[6] intravenously and urine was collected for the subsequent 24 hours. Only 0.5 to 1.5 per cent of the administered caffeine was excreted unchanged, indicating that the drug had undergone almost complete transformation in the body.

[5] Organ tissues are prepared for analysis by emulsification in water as described in a previous paper (Brodie *et al.*, 1947).

[6] The caffeine was administered in this and in subsequent experiments as caffeine-sodium benzoate.

Absorption of caffeine. Information was obtained concerning the part played by absorption from the gastrointestinal tract in the physiological disposition of caffeine. Plasma levels of the drug were compared after oral and intravenous administration of 7 mgm. per kgm. of caffeine in three male subjects. After oral administration, peak plasma levels of about 10 mgm. per liter occurred in about one hour after which the levels closely approximated those following the intravenous administration (fig. 1). Absorption from the gastrointestinal tract is thus rapid and essentially complete.

Distribution of caffeine in tissues. The extent to which caffeine in plasma is bound to proteins was determined by dialysis at 37°C. for 18 hours through

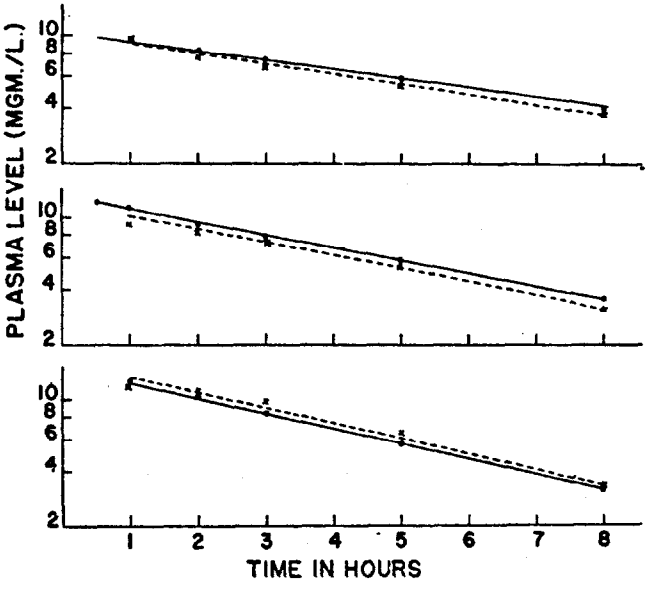

FIG. 1. Plasma levels of caffeine after the oral (solid line) and intravenous (dashed line) administration of 7 mgm. per kgm. of caffeine to three subjects.

Visking membranes against isotonic phosphate buffer at pH 7.4. In these experiments the drug was added directly to plasma. At plasma levels ranging from 9 to 45 mgm. per liter, about 15 per cent of the caffeine was found to be bound to the non-diffusible constituents of the plasma.

The distribution of caffeine was examined in representative tissues of a dog 3 hours after the intraperitoneal injection of 8½ mgm. per kgm. of the drug. The animal was sacrificed by an intravenous injection of air and the tissues were sampled immediately afterwards. Tissue water was determined by drying to constant weight at 95–100°C. The tissue concentration of caffeine was measured and calculated in terms of tissue water. The caffeine concentration in the water of various tissues was found to be nearly the same as that in plasma water (table 2), indicating that the drug is distributed in tissues in approximate proportion to their water content. The concentration of caffeine in cerebrospinal fluid was

found to be almost the same as that in plasma water, suggesting that there is little hindrance to the passage of the drug across the blood-brain barrier.

The rate of disappearance of intravenously administered caffeine from plasma. Twelve male subjects were given 7 mgm. per kgm. of caffeine, administered intravenously, and the drug plasma levels were measured at various time intervals. In all cases the logarithm of the plasma concentration when plotted against

TABLE 2

The distribution of caffeine in dog

The dog received 84 mgm. per kgm. of caffeine intravenously. The tissues were examined 3 hours after the administration of the drug.

TISSUE	CAFFEINE IN TISSUE WATER *mgm./kgm.*	RATIO: $\frac{\text{Tissue water caffeine}}{\text{Plasma water caffeine}}$
Plasma	82.7	1.0
Red blood cells	83.0	1.0
Cerebrospinal fluid	78.2	0.95
Liver	91.7	1.11
Lung	71.5	0.87
Heart	77.0	0.93
Muscle	71.0	0.86
Kidney	82.1	0.99
Spleen	73.2	0.89
Brain	77.2	0.93

TABLE 3

Plasma levels of caffeine after coffee drinking

Two cups of coffee taken at 8:00 a.m., 12 noon, 3:00 p.m., and 6:00 p.m. Blood samples were drawn at 8:00 a.m. (B_0), 9:00 a.m. (B_1), 1:00 p.m. (B_2), 7:00 p.m. (B_3), and the following morning at 8:00 a.m. (B_4). No caffeine containing beverages were taken 24 hours prior to the experiment or during the interval between B_3 and B_4.

SUBJECT	PLASMA CAFFEINE CONCENTRATION MGM./L.				
	B_0	B_1	B_2	B_3	B_4
1	0.4	2.7	3.5	4.8	0.3
2	0.9	1.5	1.9	4.0	0.2
3	0.5	0.7	1.3	4.2	0.6
4	0.4	1.9	2.8	3.9	0.7
5	0.03	1.6	2.1	3.6	0.5

time yielded a straight line indicating that caffeine disappears at a rate proportional to its plasma concentration (fig. 1, typical examples). Since negligible amounts of caffeine are excreted in urine and the drug is not localized in tissues, this decline reflects its rate of biotransformation. The biologic half-life, that is the time required for the plasma level to fall to half its value, was found to be fairly uniform from subject to subject, averaging 3.5 hours and ranging from 2.5 to 4.5 hours (12 to 22 per cent disappearance per hour).

Biotransformation of caffeine in dogs. Plasma levels and urinary excretion of caffeine were studied in dogs following the intravenous administration of 4

mgm. per kgm. of the drug. In this species also the drug was almost entirely metabolized, little or none being excreted in the urine. The biologic half-life in the dog averaged 5 hours, a value about the same as that found for man.

Accumulation of caffeine after repeated coffee drinking. In view of the widespread consumption of coffee it was of interest to determine whether caffeine accumulates in the body after the repeated drinking of coffee. Plasma levels of caffeine were measured in five human subjects who has consumed eight cups of coffee[7] over a period of seven hours. It is apparent that after the repeated intake of coffee considerable amounts of caffeine accumulate in the body (table 3). The total amount of caffeine in the body may be calculated by multiplying the total body water by the concentration of drug in plasma water, since it has been shown (*vide supra*) that caffeine is approximately evenly distributed in body water. Assuming a total body water content of 55 per cent (Soberman *et al.*, 1950) it can be estimated that about 180 mgm. of the drug were present in the body one hour after the last cup of coffee. This amount of caffeine is approximately equivalent to a therapeutic dose of the drug. There was no day to day accumulation of caffeine, as shown by the virtual disappearance of the drug by the following morning.

SUMMARY

A sensitive method for the estimation of caffeine in biological material is described. The method is specific in that it does not include transformation products of caffeine in the measurement.

Caffeine is rapidly and essentially completely absorbed from the gastrointestinal tract of man.

Caffeine is almost entirely transformed in man, only about 1 per cent being excreted in the urine. The rate of biotransformation is fairly uniform from subject to subject, the average half-life being 3.5 hours (15 per cent metabolized per hour). The metabolic rate of transformation in dogs is about the same as in man.

Caffeine is distributed in various tissues in approximate proportion to their water content. The drug passes rapidly into the central nervous system.

Although a considerable amount of caffeine accumulates in the body of moderately heavy coffee drinkers during the day, there is no day to day accumulation of the drug.

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REFERENCES

BRODIE, B. B., UDENFRIEND, S., AND BAER, J. E.: J. Biol. Chem., **168:** 299, 1947.

FISHER, R. S., ALGERI, E. J., AND WALKER, J. T.: J. Biol. Chem., **179:** 71, 1949.

ISHLER, H. H., FINUCANE, T. P., AND BORKER, E.: Anal. Chem., **20:** 1162, 1948.

KUNZ, A. F.: Biochem. Z., **275:** 270, 1935.

SOBERMAN, R., BRODIE, B. B., LEVY, B., AXELROD, J., HOLLANDER, V., AND STEELE, J. M.: J. Biol. Chem., **179:** 31, 1949.

TANAKE, U., AND OHKUBO, Y.: J. Coll. Agr., Tokyo Imp. Univ., **14:** 153, 1937.

[7] Soluble coffee was used in these experiments. Each cup of coffee contained about 80 mgm. of caffeine.